BIOSYNTHESIS OF BIOBUTANOL FROM OIL PALM FROND JUICE BY *Clostridium acetobutylicum*

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Tenaga dari sumber biomas menjadi semakin penting, kerana ia boleh digunakan untuk menggantikan sumber tenaga konvensional. Industri kelapa sawit Malaysia menjana banyak biomas lignoselulosa yang menyebabkan masalah pelupusan. Oleh itu, jus kelapa sawit (OPF) diperkenalkan sebagai medium dalam proses penapaian Aseton-Butanol-Ethanol (ABE) untuk menghasilkan biobutanol oleh *Clostridium acetobutylicum* ATCC 824. Kajian ini bertujuan untuk mengkaji potensi jus OPF sebagai medium dengan membandingkan pengeluaran butanol oleh gula tiruan sebagai percubaan pada awal kajian. Selanjutnya, kajian ini meneruskan objektif kedua dan ketiga untuk menilai dan mengoptimumkan faktor yang mempengaruhi penapaian ABE oleh *C. acetobutylicum* ATCC 824. Kandungan gula dalam jus OPF ditentukan untuk mengenal pasti kepekatan gula pada awal proses penapaian. Kawalan gerak balas permukaan (RSM) digunakan untuk menyaring dan mengoptimumkan penghasilan butanol. Jumlah gula dalam jus OPF adalah 68.58 g/L, dengan nilai glukosa, sukrosa dan fruktosa 48.19 g/L, 8.48 g/L dan 11.91 g/L. Hasil butanol dihasilkan oleh gula sintetik dalam eksperimen kawalan seolah-olah sama berbanding dengan penapaian dalam jus OPF, dengan hanya 11.25% lebih tinggi daripada jus OPF. Dalam analisis faktorial, kepekatan ekstrak yis adalah faktor tertinggi yang mempengaruhi proses penapaian dengan sumbangan 8.20%. Faktor-faktor sumbangan tertinggi kedua dan ketiga adalah saiz inokulum dan suhu inkubasi. Tiga faktor ini dioptimumkan menggunakan RSM. Keadaan optimum penapaian itu didapati pada 10% saiz inokulum, suhu pengeraman 37°C dan kepekatan ekstrak yis 5.5 g / L. Secara keseluruhan, penapaian ABE untuk menghasilkan biobutanol menggunakan jus OPF oleh *C. acetobutylicum* ATCC 824 mempunyai potensi tinggi yang kemudiannya boleh digunakan sebagai medium komersial dalam industri biominyak.

ABSTRACT

Energy from biomass resources is becoming increasingly important, since it can be used to partly displace conventional sources of energy. The Malaysian oil palm industry generates huge quantities of lignocellulosic biomass which created a major disposal problem. Therefore, oil palm frond (OPF) juice was introduced as a substrate in Acetone-Butanol-Ethanol (ABE) fermentation to produce biobutanol by *Clostridium acetobutylicum* ATCC 824. This study aims to investigate the potential of OPF juice as a substrate for the butanol production. During preliminary study, the production in OPF juice was compared with synthetic sugar as control experiment. Next, this study proceeds with the second and third objective to screen and optimize the factors affecting ABE fermentation by *C. acetobutylicum* ATCC 824. Sugar content in OPF juice was determined to identify the initial sugar concentration for the fermentation. Response Surface Methodology (RSM) was employed to screen and optimize the butanol production. The total sugar analyzed using High Performance Liquid Chromatography (HPLC) in OPF juice was 68.58 g/L, with glucose, sucrose and fructose value 48.19 g/L, 8.48 g/L and 11.91 g/L, respectively. The culture produced 9.24 g/L of biobutanol using OPF juice with 0.24 g/g biobutanol yield. Meanwhile, 10.91 g/L biobutanol produced using synthetic sugars as control experiment with 0.27 g/g biobutanol yield. The biobutanol yield produce by synthetic sugar in control experiment seem comparable to the fermentation in OPF juice, with only 11.25% higher than OPF juice. In factorial analysis, yeast extract concentration was the highest factor affecting the fermentation process with 8.20% contribution. The second and third highest contribution factors was inoculum size and incubation temperature. These three factors were then optimized using RSM. The optimum condition of the fermentation was found out at 10% inoculum size, 37°C incubation temperature and 5.5 g/L yeast extract concentration which 0.2992 g/g biobutanol yield was obtained in validation process. These experimental findings were in close agreement with the model prediction, with a difference only 9.76%. Overall, ABE fermentation to produce biobutanol using OPF juice by *C. acetobutylicum* ATCC 824 has high potential which later can be used as commercial substrate in biofuel industry.

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LIST OF SYMBOLS

g	Gram
g/g	Gram per gram
g/L	Gram per liter
g/L/h	Gram per liter per hour
hr	hour
R^2	R-squared
°C	Degree celcius
%	Percentage
v/v	Volume per volume

LIST OF ABBREVIATIONS

ABE	Acetone-Butanol-Ethanol
ANOVA	Analysis of Variance
CCD	Central Composite Design
DNS	Dinitrosalicyclic acid
DOE	Design of experiments
GC	Gas chromatography
HPLC	High performance liquid chromatography
OPF	Oil palm frond
RSM	Response surface methodology
OPT	Oil palm trunk
EFB	Empty fruit bunches
POME	Palm oil mill effluent
FFB	Fresh fruit bunches
CDW	Cell dry weight
FFD	Full factorial design
MPOB	Malaysian palm oil board
NaOH	Sodium hydroxide
HCL	Hydrochloric acid
$(NH_4)2SO_4$	Ammonium sulfate
KH_2PO_4	Monopotassium phosphate
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$Fe(NO_3)_3.9H_2O$	Iron(III) nitrate nonahydrate
$(MnSO_4.4H_2O)$	Manganese(II) sulfate tetrahydrate
ATCC	American type of culture collection
OD	Optical density
RPM	Rotation per minute
YE	Yeast extract

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